Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

THE DETERMINATION OF THE OPTIMUM AMOUNT OF ANTIGEN IN COMPLEMENT-FIXATION TESTS *

STUDIES ON COMPLEMENT FIXATION. VII

R. L. KAHN AND S. R. JOHNSON

From Bureau of Laboratories, Michigan Department of Health, Lansing, Mich.

While studying the quantitative relation between serum and antigen in Wassermann as well as specific complement-fixation tests, data was obtained which suggested a simple procedure for determining the optimum amount of antigen for these tests. In this paper we shall briefly discuss the present method of titrating antigens for complement-fixation tests, and we shall outline the proposed method based on the newer observations.

INTRODUCTION

In the report of the Medical Research Committee (Great Britain) on the Wassermann test,[1] the following three procedures were suggested for the purpose of determining more closely the strength of the Wassermann reaction of a given serum:

"(1) To test the serum against diminishing quantities of extract (antigen), keeping the amounts of the other ingredients the same.

"(2) To use diminishing amounts of serum, keeping the amounts of the other ingredients the same.

"(3) To test the serum against increasing quantities of complement, such as 3, 5, 10 and 15 doses."

Judging from procedures (1) and (2), it would appear that there exists a direct proportional relation between serum and antigen in the Wassermann test; that decreasing serum, keeping the antigen constant or decreasing antigen, keeping the serum constant, gives similar results. The wide acceptance of this view is indicated by the fact that most serologists recommend the employment of proportionally large amounts of antigen in complement-fixation tests, the assumption being that when the amounts of the various ingredients are kept the same, the larger the amount of antigen employed the stronger the reaction.

Three "titrations" form, at the present time, the basis for standardizing a given antigen for complement-fixation tests. The first titration

Received for publication July 15, 1922.

---

*For preliminary report see Proceed. Soc. for Exper. Biol. and Med., 1921, 19, p. 128.

[1] The Wassermann Test: Special Report, Series No. 14 of the Medical Research Committee, H. M. Stationery Office, London, 1918, p. 41.

has for its aim the determination of the antigenic unit or the smallest quantity of antigen which will produce complete fixation of complement in the presence of a given amount of positive serum in some standardized complement-fixation technic. The second titration determines the smallest quantity of antigen which is capable of binding complement in the absence of positive serum (anticomplementary unit), and the third titration, the smallest quantity which is capable of exerting any lytic properties on red cells (hemolytic unit). Based on the results of these titrations, one aims to employ in the tests a quantity of antigen which should contain as many antigen units as possible and at the same time be neither anticomplementary nor hemolytic in at least twice this quantity.

It is obvious that an antigen employed in complement-fixation tests should be as free as possible from anticomplementary and hemolytic properties. Whether or not, however, it can be accepted as a general rule that a larger number of antigen units represents a higher degree of sensitiveness is, in our opinion, questionable. Coca and L'Esperance[2] have indeed shown in the case of the Wassermann test that a smaller amount of lipoid antigen may, in some cases, give a stronger Wassermann reaction than a larger amount of the same antigen. And yet it is widely accepted that a high degree of concentration of antigen is essential for a high degree of sensitiveness in this reaction. Thus, Ottenberg,[3] who has recently studied various methods for determining the optimum amount of antigen for the Wassermann reaction, sums up his paper by saying: "In the Wassermann systems in which the dose of complement is either fixed (0.1 c.c.) or is two hemolytic units, . . . the more one can increase the antigen dose (and still remain below the point where nonspecific fixations are obtained) the better the results." It should be stated that in the complement-fixation system referred to in this paper, 2 hemolytic units of complement are employed.

In complement fixation with bacterial antigen the same general view prevails, namely, that the more antigen one employs in a given test the more sensitive the reaction, providing the quantity of antigen used is free from anticomplementary and hemolytic properties.

Considered from a purely theoretical point of view, the method of determining antigen units by titration with strongly positive serums is open to question. Since large variations exist in the complement-binding power of different positive serums, an antigen unit obtained with one positive serum may not be the same unit that would be obtained with a serum of different potency. Thus, it is well known that an antigen unit obtained with a serum which approaches 400 plus will contain considerably less antigenic substance than the unit obtained with a serum which just approaches 4 plus. In other words, although the implication when speaking of an antigen unit is that we are dealing with a relatively constant quantity, it may show wide variations depending on the potency of the serum employed in the determination of the unit. Furthermore, in employing a relatively large number of units, we are employing an empirical quantity rather than one which conforms with the needs of the serums tested.

---

[2] Jour. Immunol., 1916, 1, p. 129.

[3] Ibid., 1917, 2, p. 47.

Our aim should be in a given antigen titration, not to find the smallest amount which will produce fixation with a strongly positive serum and then arbitrarily use many times this amount, but rather to find, if possible, the amount of antigen which will be capable of rendering the complement-fixation reactions as sensitive as possible. Fortunately, strongly positive serums are not markedly affected by the amount of antigen employed. These serums will give strong reactions with quantities of antigen which are considerably removed from the optimum range of fixation. The problem thus narrows itself down to the determination of the amount of antigen which will be capable of enhancing the reactions with serums of moderate or weak potency. This problem forms the underlying basis of the studies reported in this paper.

EXPERIMENTS

An attempt was first made to study the quantitative relation between serum and antigen in complement-fixation tests. It was desired to find to what extent serum and antigen might be replaced by one another and still obtain the same results. Three Wassermann and three bacterial antigens were employed in these experiments. The former antigens consisted of an alcoholic extract of dried beef heart muscle previously freed from ether soluble lipoids; the same antigen, fortified with 0.4% cholesterol and a Noguchi antigen prepared from dried beef heart. The bacterial antigens consisted of B. abortus, B. mallei and B. typhosus. The complement-fixation tests were carried out as outlined in the earlier papers of this series.

The following is an outline of the first experiment, as recorded in table 1 (serum 1). A serum giving a + + + + reaction with the Wassermann test was inactivated for one-half hour at 56 C. and diluted with salt solution in the proportion of 1:10. The diluted serum was pipetted into 6 rows of 10 Wassermann tubes as follows: The first row received 0.01 c c of diluted serum, the second row, 0.02 c c, the third 0.03 c c, the fourth 0.05 c c, the fifth, 0.07 c c and the sixth, 0.1 c c of diluted serum. Alcoholic extract antigen was diluted with salt solution in the proportion of 1:50 and the mixture pipetted into each of the 6 rows of tubes. The amounts ranged from 0.01 to 0.3 c c. One-tenth c c of complement, equivalent to 2 units, was added to each tube and sufficient amount of salt solution to make a total quantity of 0.3 c c in each case. Fixation was carried out in the icebox for 4 hours. After this period 0.1 c c sheep cell suspension (5%) and 0.1 c c of amboceptor solution (2 units) were added and incubated for about 15 minutes in the water bath. At the end of this period the rack of tubes was placed in the icebox over night. The readings were recorded the following morning.

An examination of table 1 reveals several interesting phases regarding the relation of serum and antigen in complement-fixation. One may consider the 0.01 and 0.02 c c quantities of serum (serum I) as having insufficient reagin for complement binding and therefore as corresponding to negative serums. As the serum approaches the 0.1 c c quantity, it gradually gains in complement-binding power. It thus appears that the higher the concentration of complement-fixing substances in the serum the stronger the reaction. In other words, there is a direct relation between the quantity of serum employed and the strength of the reaction, which, indeed, is well established. When turning to the antigen, we find that antigenic potency and strength of the final reaction are not directly proportional to one another, except in a limited degree. This reagent appears to have a narrow range of optimum fixation for serums of weak or moderate potency. Strongly positive serums do not seem to be markedly affected by the amounts of antigen employed; quantities which are considerably removed from the optimum binding range will

TABLE 1

Amount of Antigen Giving Strongest Complement Fixation Reactions

Syphilitic Serum		Alcoholic Antigen Prepared from Beef Heart, C c									
No.	C c	0.01	0.02	0.04	0.06	0.08	0.1	0.15	0.2	0.25	0.3
I	0.01	—*	—	—	—	—	—	—	—	—	—
	0.02	—	—	—	—	—	—	—	—	—	—
	0.03	—	—	±	±	2	2	1	1	—	—
	0.05	—	—	1	1	3	3	3	2	2	±
	0.07	—	—	1	4	4	4	4	4	4	4
	0.1	—	—	2	4	4	4	4	4	4	4
II	0.01	—	—	—	—	—	—	—	—	—	—
	0.02	—	—	±	1	3	3	1	1	1	±
	0.03	—	—	1	2	3	3	3	3	3	2
	0.05	—	±	1	3	4	4	4	4	4	4
	0.07	—	±	1	3	4	4	4	4	4	4
	0.1	—	±	1	3	4	4	4	4	4	4
III	0.01	—	—	—	—	—	—	—	—	—	—
	0.02	—	—	—	—	—	—	—	—	—	—
	0.03	—	—	—	—	—	—	—	—	—	—
	0.05	—	—	1	1	1	1	—	—	—	—
	0.07	—	1	1	2	3	3	3	2	2	2
	0.1	±	3	3	4	4	4	4	4	3	3

* In this and subsequent tables, 4 = ++++, 3 = +++, 2 = ++, 1 = +, and — = negative.

nevertheless give 4 + reactions. In the case of weak serums, however, the amount of antigen employed appears to play an important part in the final outcome of the reaction. If a quantity of antigen outside of the optimum range is employed, such serums may show little or no complement-binding powers. According to the findings recorded in table 1, the optimum antigen range with each of the 3 serums tested is from 0.08 to 0.1 c c. It is apparent that only within this range will one obtain most sensitive reactions with this antigen.

It is of interest to note that the results obtained with each of the 3 serums in table 1 indicate also the so-called antigen unit, or smallest amount which gives complete fixation of complement with serums of

TABLE 2

Amount of Antigen Giving Strongest Complement-Fixation Reactions

Syphilitic Serum		Cholesterinized Antigen Prepared from Beef Heart, C c									
No.	C c	0.01	0.02	0.04	0.06	0.08	0.1	0.15	0.2	0.25	0.3
I	0.01	—	—	—	—	—	—	—	—	—	—
	0.02	—	—	—	—	—	—	—	—	—	—
	0.03	—	—	—	1	2	1	±	±	—	—
	0.05	—	—	1	4	4	4	3	3	1	±
	0.07	—	1	3	4	4	4	4	4	4	4
	0.1	—	1	3	4	4	4	4	4	4	4
II	0.01	—	—	—	—	—	—	—	—	—	—
	0.02	—	—	—	—	—	—	—	—	—	—
	0.03	—	—	—	—	—	—	—	—	—	—
	0.05	—	—	—	1	1	1	—	—	—	—
	0.07	—	—	1	3	3	2	2	2	2	1
	0.1	—	—	2	4	4	4	4	4	3	2
III	0.01	—	—	1	1	2	2	1	1	1	—
	0.02	—	—	2	2	4	3	3	2	1	—
	0.03	—	±	2	3	4	4	4	3	3	3
	0.05	—	±	3	4	4	4	4	4	4	4
	0.07	—	1	3	4	4	4	4	4	4	4
	0.1	—	1	3	4	4	4	4	4	4	4

high potency. Thus, in the case of serums I and III, the unit of antigen is 0.06 c c; in serum II, 0.08 c c. The fact that the optimum amount of antigen approaches the antigen unit, would indicate that the amount of antigen which is highly sensitive with weak serums does not necessarily consist of a large number of units. Two units, or better still 1½ units, would seem to be the amount of antigen for sensitive reactions. It is not unlikely that many of the difficulties which occur from time to time in connection with complement-fixation tests are due to the employment of relatively large amounts of antigen. For, aside from the inhibitory effect of such antigen quantities on some weakly positive serums, foreign elements are introduced which are capable of nonspecific fixation of complement.

Tables 2 and 3 give the results obtained with a cholesterinized and Noguchi antigen. Both of these antigens were diluted 1 : 75 with normal salt solution. These dilutions were based on previous experience gained with these antigens. The amount of salt solution to be employed for diluting a given extract antigen before titrating it depends largely on the way the antigen has been prepared. Thus, an extract prepared from wet heart muscle might be diluted 1 : 10 to 1 : 30 with saline, whereas if the extract is prepared from dried muscle, it might be diluted about 1 : 50 to 1 : 75. A glance at table 2 and 3 will indicate that the findings obtained with the cholesterinized and Noguchi antigens are practically the same as those obtained with the alcoholic antigen.

TABLE 3

AMOUNT OF ANTIGEN GIVING STRONGEST COMPLEMENT-FIXATION REACTION

Syphilitic Serum		Noguchi Antigen Prepared from Beef Heart, C c									
No.	C c	0.01	0.02	0.04	0.06	0.08	0.1	0.15	0.2	0.25	0.3
I	0.01	—	—	—	—	—	—	—	—	—	—
	0.02	—	—	±	1	1	1	±	±	—	—
	0.03	—	—	1	1	1	1	1	±	—	—
	0.05	—	1	1	1	2	2	1	1	1	1
	0.07	—	1	2	3	3	3	3	3	2	2
	0.1	—	2	4	4	4	4	4	4	4	4
II	0.01	—	—	—	—	—	—	—	—	—	—
	0.02	—	—	1	1	1	1	1	±	—	—
	0.03	1	1	2	2	3	3	2	2	1	1
	0.05	—	—	2	2	3	3	3	2	2	1
	0.07	1	1	2	2	3	3	3	3	3	2
	0.1	1	2	3	4	4	4	4	4	4	4
III	0.01	—	—	—	1	1	1	1	1	—	—
	0.02	—	—	2	2	4	4	4	4	4	1
	0.03	—	—	4	4	4	4	4	4	4	4
	0.05	2	3	4	4	4	4	4	4	4	4
	0.07	2	3	4	4	4	4	4	4	4	4
	0.1	2	3	4	4	4	4	4	4	4	4

From 0.08 to 0.1 c c appears to be the optimum amount of antigen in both cases.

It should be stated that any procedure for finding the optimum amount of antigen will necessarily apply only to a given complement-fixation system. These tables, therefore, should be looked on as suggestive outlines only. Different workers will carry out different antigen titrations depending on their complement-fixation technic, just as different workers titrate complement differently although for the same end. There will be workers, for example, who will first determine the antigen unit in the usual manner and then by a simple titration find the number of units giving optimum results with weak serums. Then again there may be workers who, although following the original outline, will not find it necessary to employ 6 rows of tubes in a given antigen titration, but instead, limit themselves to 3 rows. The important point to keep in mind is that it is desirable not to find the amount of antigen which will give fixation with a serum which is rich in complement fixing substances, but rather with a serum which contains as few of these substances as possible.

When turning to specific complement-fixation tests carried out with bacterial antigens, we find, as was true in the Wassermann test, that there is a direct relation between the quantity of positive serum employed and the strength of the reaction. In the case of the antigen, however, we do not find an optimum range of fixation analogous to that shown by the Wassermann antigens. Furthermore, increasing the amount of bacterial antigen keeping the serum constant has only a limited effect on the final results. Thus, an amount of serum giving, let us say, a 2 + reaction with 0.05 c c of antigen, will be likely to give the same reaction with as much as 0.3 c c of antigen.

TABLE 4

RELATION OF SERUM TO ANTIGEN IN SPECIFIC COMPLEMENT-FIXATION TESTS

Immune Bovine Serum		B. abortus Antigen, C c									
No.	C c	0.01	0.02	0.04	0.06	0.08	0.1	0.15	0.2	0.25	0.3
I	0.01	—	—	—	—	—	—	—	—	—	—
	0.02	—	—	—	—	—	—	—	—	—	—
	0.03	—	—	—	—	—	—	—	—	—	—
	0.05	—	—	±	2	3	4	4	4	4	4
	0.07	—	—	2	2	3	4	4	4	4	4
	0.1	—	—	2	2	4	4	4	4	4	4
II	0.01	—	—	±	2	3	4	4	4	4	4
	0.02	—	—	±	3	4	4	4	4	4	4
	0.03	—	—	±	3	3	4	4	4	4	4
	0.05	—	—	±	3	3	4	4	4	4	4
	0.07	—	—	2	2	4	4	4	4	4	4
	0.1	—	—	2	2	4	4	4	4	4	4
III	0.01	—	—	—	1	2	2	2	3	3	3
	0.02	—	—	1	2	2	3	4	4	4	4
	0.03	—	—	1	2	3	4	4	4	4	4
	0.05	—	—	1	2	3	4	4	4	4	4
	0.07	—	1	2	3	4	4	4	4	4	4
	0.1	—	1	2	3	4	4	4	4	4	4

The first experiments were carried out with an abortion antigen and 3 immune bovine serums. The antigen was diluted 1 : 30 with salt solution and the titration carried out as with the extract antigens and syphilitic serums. A glance at table 4 will reveal that increasing the quantity of serum increases the strength of the reactions, whereas increasing the quantity of antigen affects the reactions in only a limited degree. It will be noted also, as in the case of the Wassermann reaction, that weak serums are far more susceptible to the antigen quantity than strong serums, the latter showing marked fixation with a wide range of antigen quantities.

TABLE 5

RELATION OF SERUM TO ANTIGEN IN SPECIFIC COMPLEMENT-FIXATION TESTS

Immune Rabbit Serum		B. mallei Antigen, C c									
No.	C c	0.01	0.02	0.04	0.06	0.08	0.1	0.15	0.2	0.25	0.3
I	0.01	—	—	—	—	—	—	—	—	—	±
	0.02	—	—	—	—	—	—	—	—	—	±
	0.03	—	—	—	1	3	4	4	4	4	4
	0.05	—	—	1	2	3	4	4	4	4	4
	0.07	—	—	1	2	3	4	4	4	4	4
	0.1	—	±	1	3	3	4	4	4	4	4
II	0.01	—	—	—	1	1	1	1	1	1	1
	0.02	—	—	1	3	3	3	3	3	3	3
	0.03	—	—	1	4	4	4	4	4	4	4
	0.05	—	—	1	4	4	4	4	4	4	4
	0.07	—	1	3	4	4	4	4	4	4	4
	0.1	—	1	3	4	4	4	4	4	4	4
III	0.01	—	—	—	—	—	—	—	—	—	—
	0.02	—	—	—	—	—	—	—	—	—	—
	0.03		—	—	1	1	1	1	2	2	2
	0.05	—	—	1	2	4	4	4	4	4	4
	0.07	—	—	3	3	4	4	4	4	4	4
	0.1	—	—	3	4	4	4	4	4	4	4

TABLE 6

RELATION OF SERUM TO ANTIGEN IN SPECIFIC COMPLEMENT-FIXATION TESTS

Immune Rabbit Serum		B. typhosus Antigen, C c									
No.	C c	0.01	0.02	0.04	0.06	0.08	0.1	0.15	0.2	0.25	0.3
I	0.01	—	—	—	—	—	—	—	—	—	—
	0.02	—	—	—	—	—	—	—	—	—	—
	0.03	—	—	1	1	1	1	1	1	1	1
	0.05	—	±	2	2	2	2	2	2	2	2
	0.07	—	1	3	3	3	3	3	3	3	3
	0.1	—	2	4	4	4	4	4	4	4	4
II	0.01	—	—	1	1	1	1	1	1	1	1
	0.02	—	—	1	2	2	2	2	2	2	2
	0.03	—	—	1	2	2	2	2	2	2	2
	0.05	—	±	2	3	3	3	3	3	3	3
	0.07	—	1	4	4	4	4	4	4	4	4
	0.1	—	2	4	4	4	4	4	4	4	4

The same general findings were obtained with glanders and typhoid antigens and specific rabbit serums. These antigens were diluted 1 : 30 with salt solution and the titrations carried out as in the previous cases. Slightly stronger reactions due to increasing the antigen quantities are observed here and there, but, as a whole, the effect is comparatively small.

A glance at tables 4, 5 and 6 indicates that the amounts of bacterial antigen which are likely to give optimum results in complement-fixation tests are not as clearly defined as in the case of the alcoholic extract antigens. Indeed, there appears to exist no optimum range of fixation with these bacterial antigens. Yet, a titration of antigen such as outlined in these tables, seems to us capable of giving a better indication of the amount of antigen to employ in the tests than the old method of determining an antigen unit with some positive serum. The antigen quantity desired is the one giving most sensitive reactions with weakly positive serums and not an arbitrary number of antigen units. Assuming that 0.1 c c of abortion antigen approaches the most sensitive amount with serums of weak potency (table 4), we would say that the employment of 0.15 or 0.2 c c would be more than ample for complement-fixation tests. The same is true with the glanders and typhoid antigens. It would appear that increasing the amounts unduly would not necessarily render the reactions more sensitive. It might, on the other hand, increase nonspecific fixation of complement. It is not unlikely that the avoidance of excessive amounts of antigen in complement-fixation tests will tend to reduce the difficulties occasionally encountered due to anticomplementary and hemolytic properties of a given antigen, since these properties usually accompany high antigenic concentrations.

It should be stated also that the aim of the antigen titrations outlined in this paper is to find only the optimum quantities capable of producing the most sensitive complement-fixation reactions. These titrations refer therefore to the specific binding powers of antigen. The anticomplementary and hemolytic properties of a given antigen must be tested separately in each case.

DISCUSSION

The widely accepted view that serum and antigen are interchangeable in complement-fixation tests, is not entirely correct. Increasing serum, keeping the antigen constant, increases the strength of the reaction. But increasing antigen, keeping the serum constant, does not in the same degree increase the strength of the reaction. Indeed the quantitative rôle of antigen in the Wassermann test appears to be different from its rôle in specific complement-fixation tests. In the former case we appear to have an optimum range of antigen capable of bringing forth most sensitive reactions. Increasing the amount of antigen beyond this range will result in extremely weak or negative reactions. This particularly applies to serums of weak potency. In the case of specific complement-fixation tests, the antigens do not give any indication of possessing an optimum range of fixation. Increasing the amount of antigen will, up to a limited degree, increase the strength of the reaction; increasing the antigen beyond this degree does not seem to affect the reaction.

It is of interest that we have here another significant difference between complement fixation with bacterial antigens and specific serums and complement fixation with alcoholic extract antigens and syphilitic serums. The comparative thermostability of specific complement-fixing substances and thermolability of the similar substances present in the serum of syphilis, has been pointed out in an earlier paper of this series.[4] It would appear that there exist inherent biologic differences between specific complement-fixation tests and the nonspecific test which is exemplified in the Wassermann reaction.

Coming back to the determination of the optimum amount of antigen in the Wassermann test, the following observation made by one of us in connection with a recently developed precipitation test for syphilis,[5] appears to throw further light on this problem. This precipitation test essentially consists of mixing a given quantity of especially prepared and diluted extract antigen with 6 times the amount of syphilitic serum, and observing precipitates after incubation. While studying the quantitative relation between serum and antigen in this test, it was early observed that it is of the utmost importance to employ the proper amount of antigen and that an excess of this reagent inhibits the precipitation reaction, in many cases. Thus, a 6: 1 proportion of serum to antigen gives good results. If the amount of antigen is gradually increased beyond this 6: 1 proportion, weaker reactions are obtained. Equal parts of serum and antigen frequently inhibits the reaction. This is particularly true in the case of weak serums.

These observations are recorded because it appears to us not unlikely that the same phenomenon which tends to inhibit the precipitation reaction, when an excess of antigen is employed, inhibits also the complement-fixation reaction. The nature of this phenomenon, we are not, at the present time, prepared to explain. We feel, however, that the findings recorded in this paper with reference to the quantitative rôle of antigen in the complement-fixation reaction combined with the analogous findings in the precipitation reaction clearly indicate that an excessive amount of antigen should be especially avoided in complement fixation in syphilis.

---

[4] Kahn, R. L.; Johnson, S. R., and Boyd, A. G.: Jour. Infect. Dis., 1921, 29, p. 639.

[5] Kahn, R. L.: Arch. Dermat. & Syph., 1922, 5, pp. 570 and 734.

With regard to complement fixation with bacterial antigens, large amounts of antigen, it appears, may also best be avoided, first, because such amounts are not necessary for sensitive reactions; second, because such amounts may lead to nonspecific fixation of complement.

SUMMARY

Studies on the quantitative relation between serum and antigen in the Wassermann as well as specific complement-fixation tests led to the envolvement of a simple titration method for the determination of the optimum amount of antigen for these tests. The widely employed method of titrating antigen for complement-fixation tests consists of determining the so-called unit or smallest amount which will produce complete fixation of complement with a strongly positive serum and employing in the tests as many units as possible, providing the final amount is free from anticomplementary and hemolytic properties. This method is, in our opinion, based on the wrong assumption that the greater the concentration of antigen, keeping the other constituents the same, the stronger the reaction.

It was observed that with alcoholic extract antigens and syphilitic serums there exists an optimum range of antigen which will give complement-fixation reactions of high sensitiveness with comparatively weak serums. This optimum antigen range may be readily determined with each antigen by a simple titration indicated in the text. This titration also indicates that the employment of excessive amounts of antigen may render weak or moderate reactions, negative.

It was further observed that with bacterial antigens and specific immune serums similar antigen titrations did not disclose any optimum range of fixation for these tests. It could be readily discerned, however, that there is no advantage in employing excessive amounts of bacterial antigens. There appears, on the other hand, to be an important disadvantage since unnecessarily large amount of antigen may lead to nonspecific absorption of complement.

CONCLUSIONS

In titrating an antigen for complement-fixation tests, the aim should be to find that amount of antigen which will give reactions of high sensitiveness with weakly positive serums. Only these serums are markedly affected by the amount of antigen; the strongly positive serums show complement-fixation powers with a comparatively wide range of antigen quantities.

The employment of excessive amounts of antigen in the Wassermann, as well as specific complement-fixation tests, should be avoided. Such amounts might lead to false negative reactions in the former test and nonspecific complement fixation in the latter tests.